METHOD #: 420.1	Approved for NPDES (Editorial Revision 1978)
TITLE:	Phenolics (Spectrophotometric, Manual 4-AAP With Distillation)
ANALYTE:	Phenolics :
INSTRUMENTATION:	Spectrophotometer
STORET No.:	32730

1.0 Scope and Application

1.1 This method is applicable to the analysis of drinking, surface and saline waters, domestic and industrial wastes.

1.2 The method is capable of measuring phenolic materials at the 5 μg/L level when the colored end product is extracted and concentrated in a solvent phase using phenol as a standard.

1.3 The method is capable of measuring phenolic materials that contain more than 50 μg/L in the aqueous phase (without solvent extraction) using phenol as a standard.

1.4 It is not possible to use this method to differentiate between different kinds of phenols.

2.0 Summary of Method

2.1 Phenolic materials react with 4-aminoantipyrine in the presence of potassium ferricyanide at a pH of 10 to form a stable reddish-brown colored antipyrine dye. The amount of color produced is a function of the concentration of phenolic material.

3.0 Comments

3.1 For most samples a preliminary distillation is required to remove interfering materials.

3.2 Color response of phenolic materials with 4-amino antipyrine is not the same for all compounds. Because phenolic type wastes usually contain a variety of phenols, it is not possible to duplicate a mixture of phenols to be used as a standard. For this reason phenol has been selected as a standard and any color produced by the reaction of other phenolic compounds is reported as phenol. This value will represent the minimum concentration of phenolic compounds present in the sample.

4.0 Sample Handling and Preservation

4.1 Biological degradation is inhibited by the addition of 1 g/L of copper sulfate to the sample and acidification to a pH of less than 4 with phosphoric acid. The sample should be kept at 4°C and analyzed within 24 hours after collection.

5.0 Interference

5.1 Interferences from sulfur compounds are eliminated by acidifying the sample to a pH of less than 4 with H_3PO_4 and aerating briefly by stirring and adding $CuSO_4$.

5.2 Oxidizing agents such as chlorine, detected by the liberation of iodine upon acidification in the presence of potassium iodide, are removed immediately after sampling by the addition of an excess of ferrous ammonium sulfate (7.10). If chlorine is not removed, the phenolic compounds may be partially oxidized and the results may be low.

6.0 Apparatus

6.1 Distillation apparatus, all glass consisting of a 1 liter pyrex distilling apparatus with Graham condenser.
6.2 pH meter.
6.3 Spectrophotometer, for use at 460 or 510 nm.
6.4 Funnels.
6.5 Filter paper.
6.6 Membrane filters.
6.7 Separatory funnels, 500 or 1,000 mL.
6.8 Nessler tubes, short or long form.

7.0 Reagents

7.1 Phosphoric acid solution, 1 + 9: Dilute 10 mL of 85% H_3PO_4 to 100 mL with distilled water.
7.2 Copper sulfate solution: Dissolve 100 g $CuSO_4 \cdot 5H_2O$ in distilled water and dilute to 1 liter.
7.3 Buffer solution: Dissolve 16.9 g NH_4Cl in 143 mL conc. NH_4OH and dilute to 250 mL with distilled water. Two mL should adjust 100 mL of distillate to pH 10.
7.4 Aminoantipyrine solution: Dissolve 2 g of 4AAP in distilled water and dilute to 100 mL.
7.5 Potassium ferricyanide solution: Dissolve 8 g of $K_3Fe(CN)_6$ in distilled water and dilute to 100 mL.
7.6 Stock phenol solution: Dissolve 1.0 g phenol in freshly boiled and cooled distilled water and dilute to 1 liter. 1 mL = 1 mg phenol.
7.7 Working solution A: Dilute 10 mL stock phenol solution to 1 liter with distilled water. mL = 10 μg phenol.
7.8 Working solution B: Dilute 100 mL of working solution A to 1000 mL with distilled water. 1 mL = 1 μg phenol.
7.9 Chloroform
7.10 Ferrous ammonium sulfate: Dissolve 1.1 g ferrous ammonium sulfate in 500 mL distilled water containing 1 mL conc. H_2SO_4 and dilute to 1 liter with freshly boiled and cooled distilled water.

8.0 Procedure

8.1 Distillation

8.1.1 Measure 500 mL sample into a beaker. Lower the pH to approximately 4 with 1 + 9 H_3PO_4 (7.1), add 5 mL $CuSO_4$ solution (7.2) and transfer to the distillation apparatus. Omit adding H_2PO_4 and $CuSO_4$ if sample was preserved as described in 4.1.

8.1.2 Distill 450 mL of sample, stop the distillation, and when boiling ceases add 50 mL of warm distilled water to the flask and resume distillation until 500 mL have been collected.

8.1.3 If the distillate is turbid, filter through a prewashed membrane filter.

8.2 Direct photometric method

8.2.1 Using working solution A (7.7), prepare the following standards in 100 mL volumetric flasks.

mL of working solution A	Conc. μg/L
0	0.0
0.5	50.0
1.0	100.0
2.0	200.0
5.0	500.0
8.0	800.0
10.0	1000.0

8.2.2 To 100 mL of distillate or an aliquot diluted to 100 mL and/or standards, add 2 mL of buffer solution (7.3) and mix. The pH of the sample and standards should be 10 ± 0.2.

8.2.3 Add 2.0 mL aminoantipyrine solution (7.4) and mix.

8.2.4 Add 2.0 mL potassium ferricyanide solution (7.5) and mix.

8.2.5 After 15 minutes read absorbance at 510 nm.

8.3 Chloroform extraction method

8.3.1 Using working solution B (7.8), prepare the following standards. Standards may be prepared by pipetting the required volumes into the separatory funnels and diluting to 500 mL with distilled water.

mL of working solution B	Conc. μg/L
0.0	0.0
3.0	6.0
5.0	10.0
10.0	20.0
20.0	40.0
25.0	50.0

8.3.2 Place 500 mL of distillate or an aliquot diluted to 500 mL in a separatory funnel. The sample should not contain more than 25 μg phenol.

8.3.3 To sample and standards add 10 mL of buffer solution (7.3) and mix. The pH should be 1O ± 0.2.

8.3.4 Add 3.0 mL aminoantipyrine solution (7.4) and mix.

8.3.5 Add 3.0 mL potassium ferricyanide solution (7.5) and mix.

8.3.6 After three minutes, extract with 25 mL of chloroform (7.9). Shake the separatory funnel at least 10 times, let $CHCl_3$ settle, shake again 10

times and let chloroform settle again. Vent chloroform fumes into hood.

8.3.7 Filter chloroform extracts through filter paper. Do not add more chloroform. Carry out filtration in a hood. Dispose of chloroform in environmentally acceptable manner.

8.3.8 Read the absorbance of the samples and standards against the blank at 460 nm.

9.0 Calculation

9.1 Prepare a standard curve by plotting the absorbance value of standards versus the corresponding phenol concentrations.

9.2 Obtain concentration value of sample directly from standard curve.

10.0 Precision and Accuracy

10.1 Using the extraction procedure for concentration of color, six laboratories analyzed samples at concentrations of 9.6, 48.3, and 93.5 μg/L. Standard deviations were ±O.99, ±3.1 and ±4.2 μg/L, respectively.

10.2 Using the direct photometric procedure, six laboratories analyzed samples at concentrations of 4.7, 48.2 and 97.0 mg/L. Standard deviations were ±0.18, ±0.48 and ±1.58 mg/L, respectively.

Bibliography

1. Annual Book of ASTM Standards, Part 31, "Water", Standard D 1783-70, p553 (1976).
2. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p574-581, Method 510 through 510C, (1975).